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研究課題名（和文）In vitro study of the association between ALDH2 rs671 polymorphism and Acral Lentiginous Melanoma

研究課題名（英文）In vitro study of the association between ALDH2 rs671 polymorphism and Acral Lentiginous Melanoma

研究代表者

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研究成果の概要（和文）：黒色腫はALDH2rs671多型やライフスタイルの選択などの遺伝的要因の影響を受けます。この研究では特にALDH2rs671キャリアにおいて、アセトアルデヒドがメラニン合成とメラノサイトの遺伝子発現に与える影響を調べました。

アセトアルデヒド、α-Melanocyte stimulating hormone及びエタノール処置により、メラニン合成とALDH2及びTyrosinase geneの発現が増加し、酸化ストレスに関与する経路が示唆されました。これらの知見は、特にALDH2rs671の頻度が高い東アジア人において、黒色腫の遺伝的およびライフスタイルのリスク要因を強調しています。

研究成果の学術的意義や社会的意義
The study underscores the roles of acetaldehyde, ethanol levels, and specific gene expressions in melanogenesis of Melanoma. It highlights the increased risk of melanoma and Melanoma by accumulating acetaldehyde from alcohol consumption,

研究成果の概要（英文）：Melanoma,is influenced by genetic factors like the ALDH2rs671 polymorphism and lifestyle choices.This study examined acetaldehyde's effect on melanin synthesis and gene expression in melanocytes, particularly in ALDH2rs671 carriers.

Treatment with acetaldehyde,α-Melanocyte stimulating hormone, and ethanol increased melanin synthesis and ALDH2 and Tyrosinase gene expression, suggesting pathways involving oxidative stress. These findings highlight genetic and lifestyle risk factors for Melanoma, especially in East Asians with a high frequency of ALDH2rs671.

研究分野：Environmental Medicine

キーワード：Melanoma　Melanin　Acetaldehyde　Ethanol　ALDH2　rs671

1．研究開始当初の背景 (Background of study)

• **Mealnoma, Acral Lentiginous Melanoma, and association with lifestyle risk factors.** Melanoma, the most severe skin cancer, is increasingly prevalent worldwide, necessitating urgent prevention measures. Its risk is intricately tied to genetic factors, lifestyle choices, and phenotypic traits. Lifestyle factors like diet, alcohol, and tobacco use play a crucial role in elevating melanoma risk through carcinogenesis. Acral Lentiginous Melanoma (ALM), distinguished by its occurrence on non-UV-exposed skin areas such as palms, soles, and the nail apparatus. However, ALM incidence is higher in East Asia. We hypothesized that a genetic disposition unique to East Asians is responsible for the higher ALM risk. Nonetheless, there have been limited studies on the incidence and prognosis of ALM.

• ***ALDH2* rs671 polymorphism with cancer risk in East Asian populations.** Alcohol itself is not carcinogenic, but its metabolite, acetaldehyde, is known to interfere with DNA synthesis and repair and consequently increase the risk of cancer. Aldehyde dehydrogenase-2 (ALDH2) is the major enzyme to eliminate most of the acetaldehyde. *ALDH2* rs671 (*ALDH2*2*) polymorphism (Figure 1) is known to associate with the alcohol-related cancer risk in Asians, especially among the Chinese and the Japanese. *ALDH2*2* carriers with drinking habits show the highest risk. Our previous research of team leader, Matsumoto found that ethanol consumption induced increases in melanocytes numbers and skin hyperpigmentation in palms and soles of *Aldh-2* deficient mice. Furthermore, the carriers of *ALDH2*2* were found to show a positive correlation between alcohol intake and skin melanin index. These findings suggest that acetaldehyde derived from alcoholic beverages stimulates melanocytes resulting in skin hyperpigmentation.

2．研究の目的 (Purpose of the research)

We hypothesize that excessive synthesis of melanin, which is known to generate oxidative stress, is induced by acetaldehyde derived from the consumption of alcoholic beverages in people carrying the rs671.

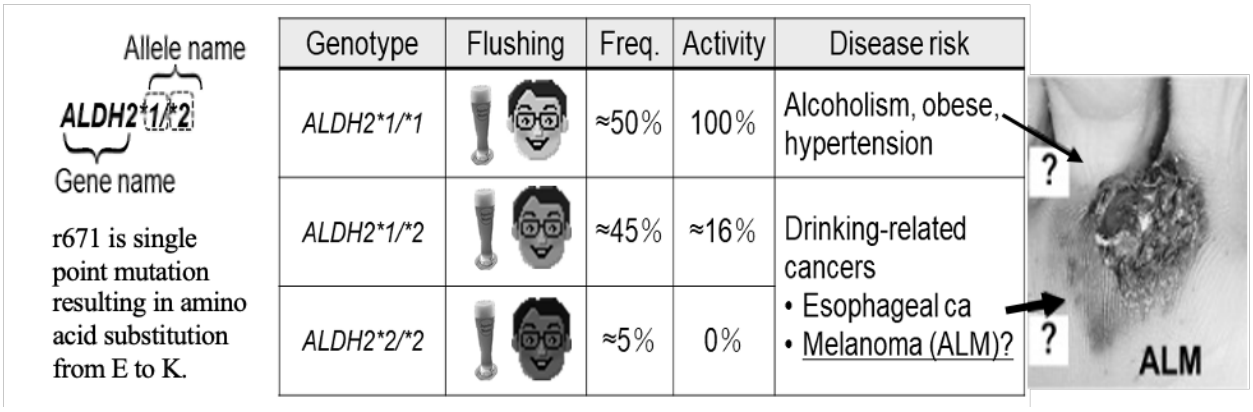

Figure 1. East Asians-specific genetic polymorphism of Aldehyde dehydrogenase 2 (ALDH2)

3．研究の方法 (Research methods)

- **Cell culture and Treatments.** The human epithelial melanocytes (HEM) cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 5% penicillin/streptomycin; HEM cells were cultured in the special culture medium at 37°C in the presence of 5% CO_2. After a primary culture from frozen stocks in 10-cm dishes, the cells were seeded at densities of 0.5×10^5, 1×10^5, 2×10^5, 3×10^5 cells/ well in 6-well-plates and incubated for 6 and 24 hours. The optimal cell density for a 6-well plate was determined to be 1×10^5 cells, achieving 80% confluency after 24 hours.

- **Treated cells with acetaldehyde, ethanol, and α-MSH.** Following 80% confluency, cells were treated with different concentration of acetaldehyde (10,000, 1,000, 100, 10 μM), ethanol (0.01%, 0.05%, 0.10%, 0.50%, 1%) , and α-MSH (alpha melanocyte stimulating hormone; 10^3 and 10^4 nM) in two sets of triplicates of HEM.

- **Melanin quantification.** Intracellular melanin was measured using HPLC and colorimetric assays with a synthetic melanin standard curve (Figure 2).

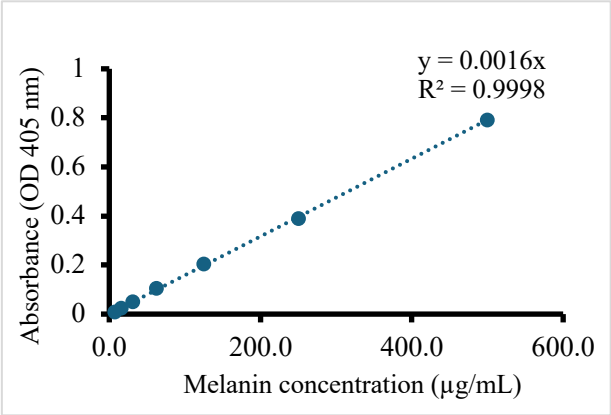

Figure 2. Standard curve for synthetic melanin

- **Morphological Observations.** Observed and documented cell shape, size, and pigmentation changes under light microscopy.

- **Gene Expression Analysis:** Total RNA was extracted using Sepazol RNAI Super G for high-quality RNA. cDNA was synthesized using 5xFastGene Scriptase II ReadyMix (LS64) for gene expression analysis. ALDH2, TYR, MITF gene expressions were assessed by qPCR, normalized to GAPDH.

- **Statistical analysis.** Each experiment was performed in two sets of triplicates using at least three independent cultures, yielding comparable results. Data are reported as the means ± SD. ANOVA and Student's *t*-test were used to compare differences between groups. $p<0.05$ was considered statistically significant.

4．研究成果 (Results)

Activation and suppression of melanogenesis. Human Epithelial Melanocytes (HEM) (1×10^5 cells) were treated with 10,000 μM acetaldehyde, 0.01% ethanol, and α-MSH (1,000 and 10,000 nM). The intracellular melanin content of the melanocytes was measured to assess the effects of different treatments on melanogenesis at two incubation periods: 6 hours and 24 hours. Preliminary data indicate that a 6-hour incubation with 10,000 μM acetaldehyde stimulates melanin synthesis, while 0.01% ethanol suppresses it (Figure 3).

Melanin content was significantly increased 10,000 μM acetaldehyde stimulates melanin synthesis as compared to untreated control (Figure 4), while suppress with 0.01% ethanol (Figure 5).

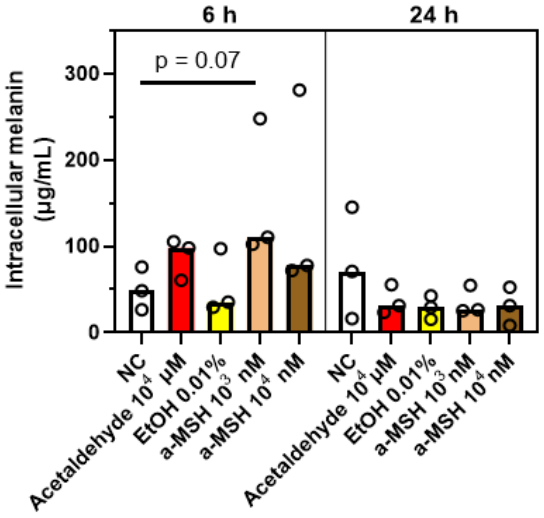

Figure 3. Melanin content. Changes in melanin contents depending on the treatment conditions and incubation times (6h and 24 h).

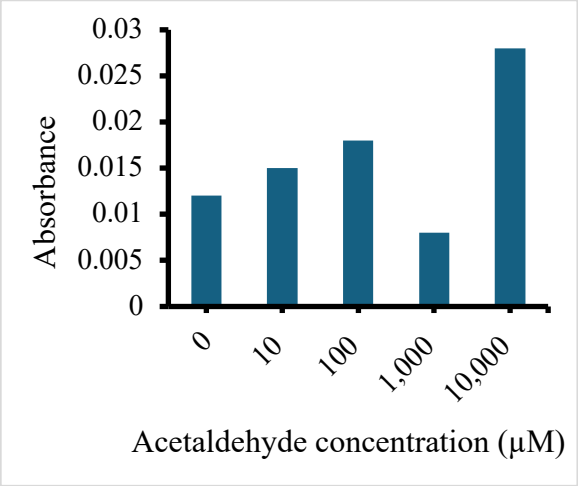

Figure 4. Effects of different concentration of acetaldehyde on melanin synthesis.

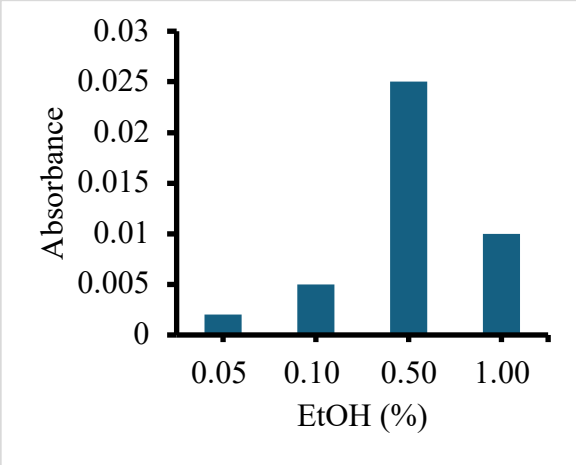

Figure 5. Effects of different percentages of ethanol (EtOH) on melanin synthesis.

Quantitative analysis showed that the *TYR* and *ALDH2* genes were significantly up-regulated in melanocytes exposed to acetaldehyde (10^4 μM) compared to the untreated control ($p < 0.05$) (Figures 6 and 7).

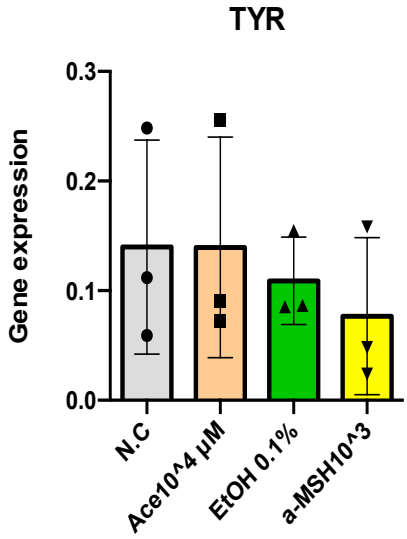

Figure 6. Changes of ALDH2 depending on depending on the treatment conditions. Ace 10^4 μM (Acetaldehyde 10^4 μM).

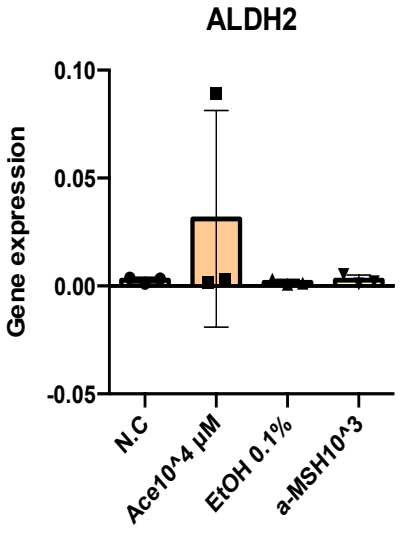

Figure 7. Changes of TYR gene depending on depending on the treatment conditions. Ace 10^4 μM (Acetaldehyde 10^4 μM).

This study shows that acetaldehyde increases melanin synthesis and upregulates ALDH2 and TYR gene expressions in melanocytes, especially in individuals with the ALDH2 rs671 polymorphism. These effects suggest acetaldehyde acts through unique pathways distinct from α-MSH and ethanol, possibly involving oxidative stress and DNA repair. Given the higher prevalence of ALDH2 rs671 in East Asians, these findings may explain the increased risk of ALM in this population, underscoring the role of genetic and lifestyle factors in melanoma development. Future research should focus on understanding how acetaldehyde influences melanocyte function and consider co-culture studies with Human Epithelial Keratinocytes (HEK) to explore microenvironmental influences on melanogenesis and melanoma progression.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Matsumoto Akiko、Hara Megumi、Ashenagar Mohammad Said、Tokiya Mikiko、Sawada Takeshi、Iwasaka Chiharu、Furukawa Takuma、Kitagawa Kyoko、Miyake Yasunobu、Hirota Yoshio	４．巻 10
２．論文標題 Variant Allele of ALDH2, rs671, Associates with Attenuated Post-Vaccination Response in Anti-SARS-CoV-2 Spike Protein IgG: A Prospective Study in the Japanese General Population	５．発行年 2022年
３．雑誌名 Vaccines	６．最初と最後の頁 1035～1035
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/vaccines10071035	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Tokiya Mikiko、Hara Megumi、Matsumoto Akiko、Ashenagar Mohammad Said、Nakano Takashi、Hirota Yoshio	４．巻 10
２．論文標題 Acceptance of Booster COVID-19 Vaccine and Its Association with Components of Vaccination Readiness in the General Population: A Cross-Sectional Survey for Starting Booster Dose in Japan	５．発行年 2022年
３．雑誌名 Vaccines	６．最初と最後の頁 1102～1102
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/vaccines10071102	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Ashenagar Mohammad Said、Matsumoto Akiko、Sakai Hironori、Tokiya Mikiko、Hara Megumi、Hirota Yoshio	４．巻 10
２．論文標題 Comparison of CLEIA and ELISA for SARS-CoV-2 Virus Antibodies after First and Second Dose Vaccinations with the BNT162b2 mRNA Vaccine	５．発行年 2022年
３．雑誌名 Vaccines	６．最初と最後の頁 487～487
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/vaccines10040487	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Tokiya Mikiko、Hara Megumi、Matsumoto Akiko、Ashenagar Mohammad Said、Nakano Takashi、Hirota Yoshio	４．巻 10
２．論文標題 Association of Vaccine Confidence and Hesitancy in Three Phases of COVID-19 Vaccine Approval and Introduction in Japan	５．発行年 2022年
３．雑誌名 Vaccines	６．最初と最後の頁 423～423
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/vaccines10030423	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　0件）

1．発表者名 M.Said Ashenagar
2．発表標題 Baseline Serum Zinc Levels Positively Associated with Humoral Response to COVID-19 Vaccine in Japanese Women
3．学会等名 Japanese Society of Hygiene (JSH)
4．発表年 2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関